REMARKS

Claims 18, 23-30, and 34-35 are pending in this application. Claims 1-17, 20-22 and 33 and 19 have been cancelled. Claim 18 has been amended to further define the lysis solution. Support for this amendment is found on page 5, lines 22-25, of the specification, and in the previous version of claim 22. Claims 31 and 32 are withdrawn. It is respectfully requested that claims 31 and 32 be rejoined in this application.

According to the Office Action, claims 18, 20-21, 24-29, 33 and 35 are rejected under 35 USC 102(b) as being anticipated by Richthoff et al. (Human Reproduction, Vol. 17, No. 12, pp. 3162-3169, 2002). This is respectfully traversed.

Anticipation requires that each and every element of the claimed invention be disclosed in a single prior art reference. *In re Paulsen*, 30 F.3d 1475, 31 USPQ 1671 (Fed. Cir. 1994). For anticipation, there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 18 USPQ2d 1001 (Fed. Cir. 1991).

The method described in Richthoff *et al.* differs from the one of the present invention and therefore is not anticipated by it. As it is mentioned in Richthoff *et al.* (page 3164, column 2, second paragraph of section "FCM SCSA"), the method followed to evaluate the integrity of the DNA is SCSA (Sperm Chromatin Structure Assay), as previously described by "Spano *et al.*, 2000" and "Evenson *et al.*, 2002". Evenson *et al.* described a review about SCSA, its clinical use for detecting sperm DNA fragmentation and compares SCSA with other techniques of DNA fragmentation evaluation. Spano *et al.* (2000) described a study of the impact of testicular and accessory sex gland function on sperm chromatin integrity as assessed by SCSA, following the methodology described in Spano *et al.* (1998), Evenson *et al.* (1991) and Evenson and Jost (1994). The methodology described in Evenson and Jost (1994) is the same that the one used in Spano *et al.* (1999), paper already cited by the Examiner Official Action of April 29, 2009. In the response to that action, arguments were presented that highlighted the differences amongst the methodology described in Spano *et al.* (1999), and therefore in Richthoff *et al.*, and the invention claimed in this application.

Likewise, in the reply to the Office Action, arguments were presented emphasizing the

advantages of having two sequential steps of denaturation and lysis (method of the present invention) instead of just one.

However to expedite prosecution of this application claim 18 has been amended to define a lysis solution. Applicants preserve all rights to file one or more divisional or continuation applications for the subject matter described in this application and not presently claimed.

The advantages arising from the treatment of the sample, which comprises two sequential steps, a denaturing step and a lysis step, each of them using a different solution, and the advantages given by the method of evaluation of the integrity of the DNA have been described previously.

A single lysis step with a single lysis solution, used in the present invention, has the effect of not affecting negatively the structure of the spermatozoids allowing for an unambiguous identification of this kind of cells with respect to other kind of cells that may be present in the sample. The use of a single lysis step, together with the single lysis solution of claim 18, also has the advantage that quality and contrast of the images obtained is very much improved giving rise to more reliable results with an improved reproducibility.

In Richthoff et al. the use of a solution, being HCl and Triton X-100 at the same time, is not capable of producing the differential effect obtained in the method of the present invention for the following reasons:

Being a single solution, the denaturant effect does not occur prior to lysis, so that differential denaturation according to the level of DNA fragmentation, as in the present invention, cannot be produced prior to lysis.

- When the components of the lysis solution exert their function to extract nuclear proteins, DNA cannot have been differentially denatured in terms of their level of fragmentation
- Therefore, there is no such differential expansion of halos according to their fragmentation (See figure 1 attached , wherein a single solution (lysis + denaturing) has been used. All cells show big halos of similar sizes, independently of the DNA fragmentation).

If a single solution (single-step treatment of the sample) is used, the procedure would not allow the denaturing components to have time to exercise their role and denature the DNA differentially depending on their fragmentation before lysis occurred. Therefore, removal of protamines and the release of halos, would occur without prior differential denaturation of DNA, which would make all the halos being equal (figure 1). That is, a solution such as that defined in Richthoff *et al.*, would produce the same halo in all sperms and would prevent the study of the level of DNA fragmentation in terms of the halo size (as in step c) of claim 18.

Therefore, first using a denaturing solution, and then a lysis solution, provides important differences that bring advantages to the method of the invention, such as direct visualization of the size of the halos (See figure 2, wherein two sequential solutions (first denaturing and then lysis solution) have been used. Here, different size halos can be clearly seen, depending on the DNA fragmentation).

Finally, the use of a single lysis solution, in absence of denaturing agent, allows for the possible sequential use in the nucleoids, of protein immunodetection techniques, such as laminins and other nuclear proteins, and the detection of RNA associated with the nuclear matrix, as the DNA spreads persisting the greatest amount possible of nuclear structure. This is important in some research issues on the structure of the sperm cell nucleus.

In terms of the evaluation step, the present invention uses a direct visual analysis, based on the measurement of the halo sizes, whereas Richthoff *et al.* use SCSA, which consists in 1) treating the sample with a denaturing detergent solution, 2) staining the sample with Acridine Orange (AO) and 3) analyzing the sample by flow cytometry (FCM). This AO staining emits a green fluorescence when it binds to double stranded DNA while, in the sperm with denatured DNA, in single strand, this fluorochrome emits a red fluorescence.

In the present invention, Wright dye or Diff-Quik can be used. These staining techniques are one of the simplest, cheapest and most routine ones in any laboratory. Its use allows for viewing the tails, as these are not visible in DNA stainings with fluorochromes used for fluorescence microscopy. It must be noted that these stainings can be very easily manipulated for obtaining the intended staining level. However, the FCM SCSA, used in Richthoff *et al.*, requires strictly following the protocol for cell preparation, staining, and measuring,

expensive flow cytometric equipment and an SCSA-trained technician.

Therefore, starting from Richthoff *et al.*, a person skilled in the art would not have developed a method as that of the present invention, with two sequential steps, one of denaturisation and another one of lysis (with a single lysis solution), and an evaluation step.

Therefore, as all elements of the claims are not disclosed in Richthoff et al. Richthoff cannot and does not anticipate claims 18, 20-21, 24-29, 33 and 35.

Therefore, it is respectfully requested that the rejection be withdrawn.

Accordingly, it is submitted that this application is in condition for allowance and favorable consideration is respectfully requested.

If any fees are due, please charge deposit account 12-0425.

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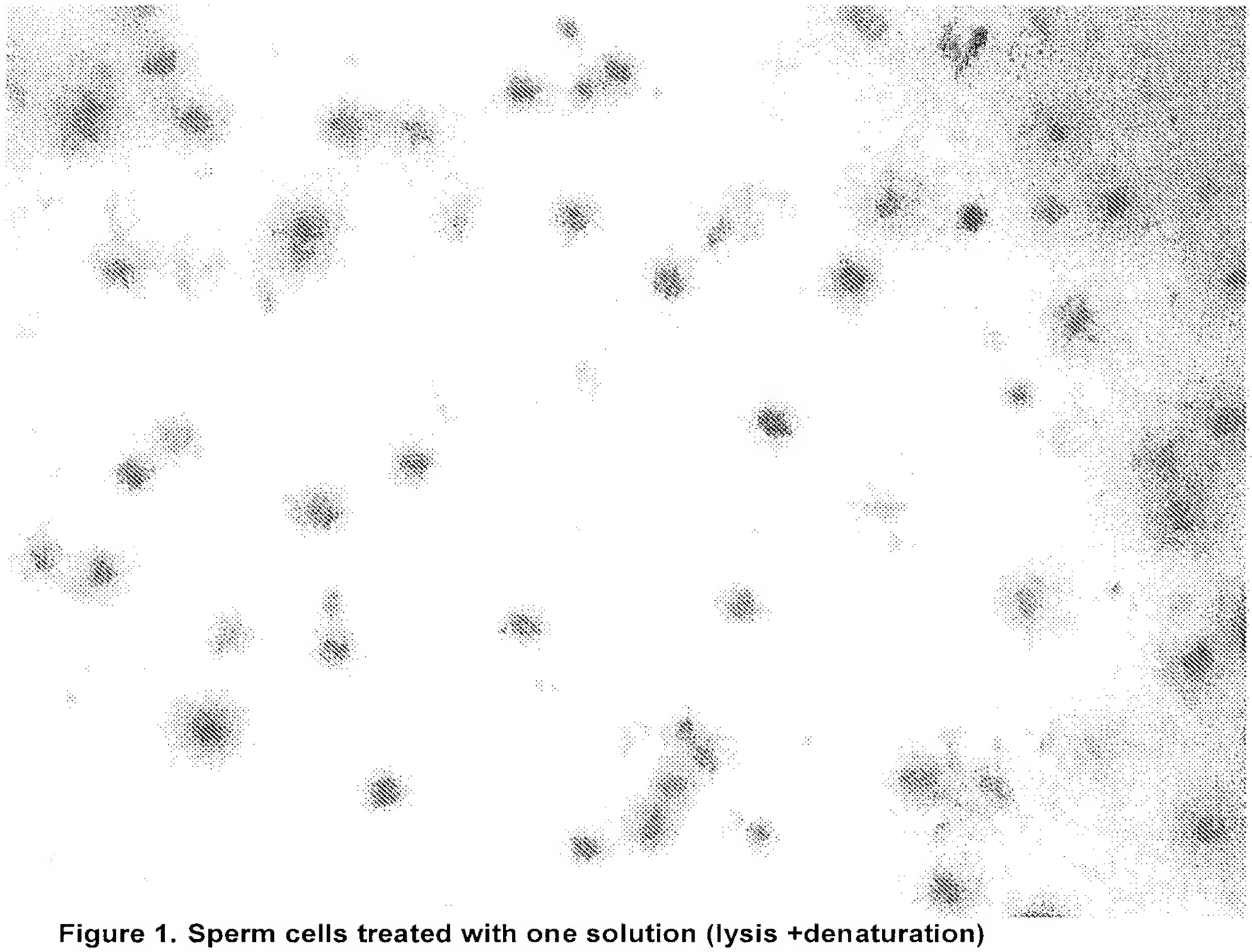

Figure 1. Sperm cells treated with one solution (lysis +denaturation)

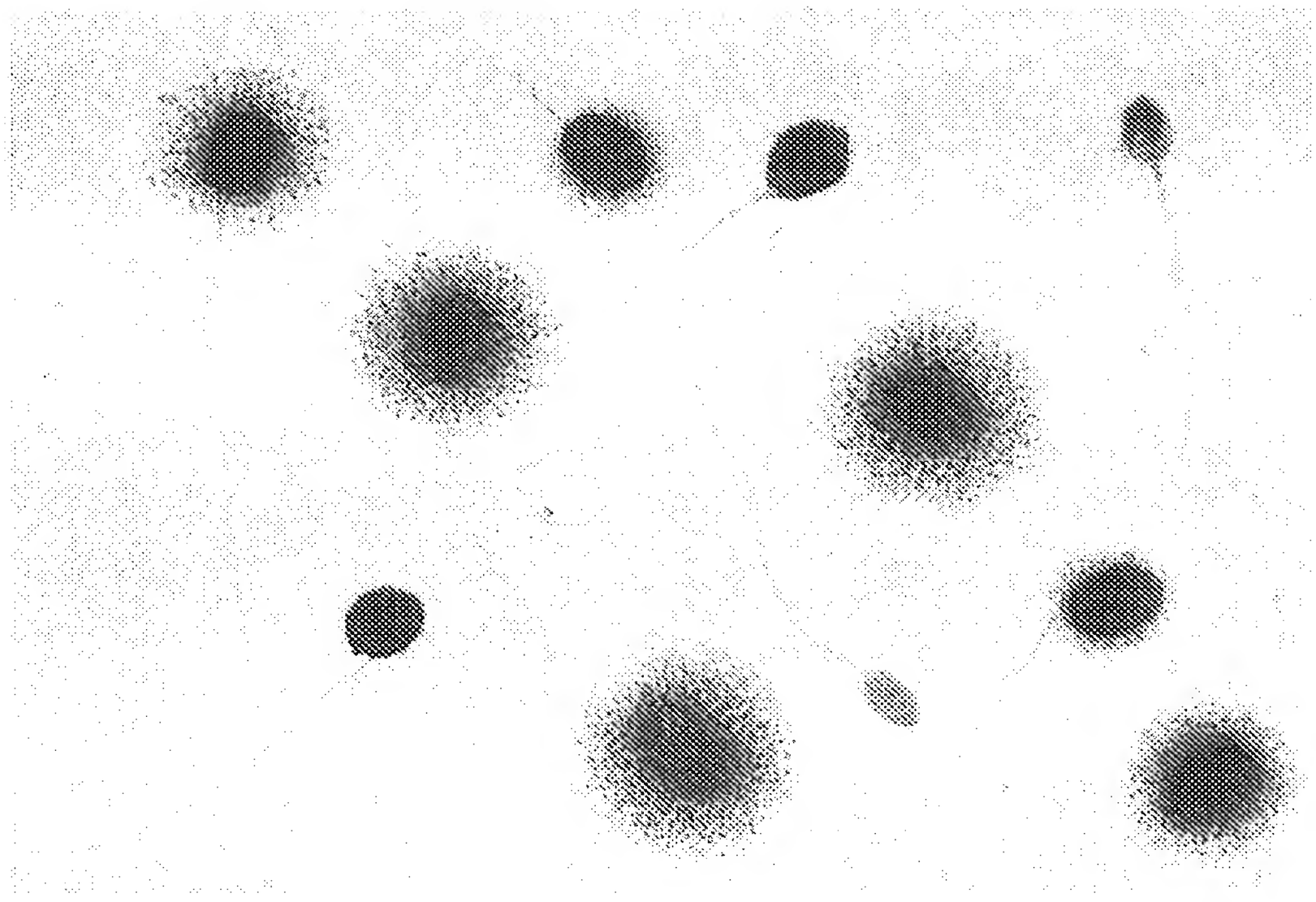

Figure 2. Sperm cells treated with two sequential solutions (1st denaturation +2nd lysis)